AD_____
(Leave blank)

Award Number: W81XWH-08-2-0157

TITLE: Glyburide – novel prophylaxis and effective treatment for ~ traumatic brain injury

PRINCIPAL INVESTIGATOR: J. Marc Simard, M.D., Ph.D.

CONTRACTING ORGANIZATION: University of Maryland, Baltimore
Baltimore, MD 21201

REPORT DATE: August 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
06/08/09	Annual	07/07/08-07/06/09

4. TITLE AND SUBTITLE
"Glyburide – novel prophylaxis and effective treatment for – traumatic brain injury"

5a. CONTRACT NUMBER
W81XWH-08-2-0157

5b. GRANT NUMBER
W81XWH-08-2-0157

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
J. Marc Simard, M.D., Ph.D.

Email: msimard@smail.umaryland.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Maryland, Baltimore
685 W. Baltimore Street
Room 6-30
Baltimore, MD 21201

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
and Materiel Command
Fort Detrich, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall subject of this project is blast-traumatic brain injury (blast-TBI) and the role of the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel in blast-TBI. The specific objectives of this project include: (1) develop a standardized rat model of blast-TBI to study the direct transcranial effects of blast on the brain, independent of indirect transthoracic effects; (2) determine the role of the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel in blast-TBI; (3) in normal human volunteers, determine the safety of the SUR1 blocker, glyburide, as it might be used as prophylaxis against blast-TBI. During the first year of this grant, we completed a key objective – the development, construction and implementation of a cranial-only blast injury apparatus (COBIA) for production of reliable, repeatable, "dose-dependent" blast-TBI, independent of transthoracic mechanisms of injury to the brain. Using COBIA, we began characterizing the pathophysiological consequences of blast-TBI, with early results suggesting the novel observation that blast-TBI can produce fatal neurogenic pulmonary edema independent of blast injury to the thorax. We also began characterizing the effect of blast-TBI on the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel. Early results suggest the novel finding that SUR1 is abundantly upregulated in neurons and oligodendrocytes, further strengthening the rationale for prophylaxis and treatment using the SUR1 inhibitor, glyburide.

15. SUBJECT TERMS
blast, traumatic brain injury, neurogenic pulmonary edema, mortality, caspase-3, beta-amylase precursor protein, sulfonylurea receptor

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	16	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The overall subject of this research project is blast-traumatic brain injury (blast-TBI) and the role of the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel in secondary injury following blast-TBI. The specific objectives of this research project may be summarized as follows: (1) develop a standardized rat model of blast-TBI, to permit study of direct transcranial effects of blast on the brain, independent of indirect transthoracic blast effects; (2) using this rat model, determine the specific role of the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel in blast-TBI, including testing whether block of SUR1 using glyburide would show a beneficial effect in blast-TBI; (3) in normal human volunteers, determine the safety of oral glyburide as it might be used as prophylaxis against blast-TBI.

BODY: This section of the report shall describe the research accomplishments associated with each task outlined in the approved Statement of Work. Data presentation shall be comprehensive in providing a complete record of the research findings for the period of the report. Provide data explaining the relationship of the most recent findings with that of previously reported findings. Appended publications and/or presentations may be substituted for detailed descriptions of methodology but must be referenced in the body of the report. If applicable, for each task outlined in the Statement of Work, reference appended publications and/or presentations for details of result findings and tables and/or figures. The report shall include negative as well as positive findings. Include problems in accomplishing any of the tasks. Statistical tests of significance shall be applied to all data whenever possible. Figures and graphs referenced in the text may be embedded in the text or appended. Figures and graphs can also be referenced in the text and appended to a publication. Recommended changes or future work to better address the research topic may also be included, although changes to the original Statement of Work must be approved by the Army Contracting Officer Representative. This approval must be obtained prior to initiating any change to the original Statement of Work.

OBJECTIVE 1: develop a standardized rat model of blast-TBI, to permit study of direct transcranial effects of blast on the brain, independent of indirect transthoracic blast effects.

Background: In humans, TBI following an area-blast is a complex multi-faceted disease. Pathophysiologically, blast-TBI results in brain edema, swelling, hemorrhage, neuronal injury and neuronal death, leading to severe neurological and neuropsychological deficits and possibly death. Even omitting well-known mechanisms of secondary, tertiary and quaternary injury, the primary injury itself is complex, and is believed to occur by way of two distinct mechanisms: (i) direct transcranial propagation of the blast wave; (ii) indirect transmittal via the vasculature following blast to the thorax. Because the pathophysiological manifestations of TBI following area-blast in the human are so complex, dissecting out the various component mechanisms and their relative contribution to the overall pathophysiological response is of great importance for future design of treatment and prophylactic measures.

Essentially all of the existing work on blast-TBI exposes the subject's total body to an area-blast. Whereas this approach appropriately simulates the real-world combat experience, it does not permit assessment of direct blast injury to the brain in isolation of indirect injury to the brain due to blast injury to other organs, especially the lungs and major vessels within the thorax. Prior to the work we are reporting here and are actively pursuing, no animal model existed to examine the direct transcranial mechanism of blast-TBI, independent of the indirect transthoracic mechanism.

As a result of these considerations, the committee reviewing our original proposal requested that we specifically focus on further developing, perfecting and assessing the direct blast-TBI model that we had utilized to obtain some of our preliminary data.

Introduction. Successful development of a usable, standardized model system for direct blast-TBI entails several sub-objectives:

(**a**) the design and fabrication of an apparatus for reproducible, graded blast directed to the cranium that would minimize exposure of the thorax;

(**b**) establishing the range of blast intensity that would be used for later biological study, including the determination of the threshold intensity for lethality; (empirically determining the "intensity-response relationship" for a given biological outcome variable is needed to allow later assessment of a given pharmacological treatment or prophylaxis in "shifting" the apparent intensity-response relationship to the right);

(**c**) characterize the basic pathophysiological, systemic response to direct sublethal blast, to ascertain the effect of brain injury on systemic variables.

Objective 1a: Cranium-only blast injury apparatus (COBIA)

During the first year of this project, we implemented several successive modifications to the original COBIA that we had used to obtain our preliminary data. We have added important improvements and features for

(i) reliable, reproducible blast injury directly to the cranium

(ii) improved targeting to the cranium, to minimize exposure of the thorax

(iii) adjustable magnitude of blast exposure, from lethal to varying levels of sublethal brain injury, allowing for "intensity-response relationship" studies.

The current version of the COBIA is shown in Fig. 1. Blast is powered by detonating a .22 caliber blank shot [Remington, power level 2 or power level 4 shots; weight of powder (mean±S.D): 0.128±0.003 or 0.179±0.003 gm, respectively] inside the firing chamber of a gun (ITW Ramset, Model RS22, Glenview IL; with piston removed). The blast is directed down the barrel of the gun, which interfaces with one of a variety of interchangeable, custom-made blast dissipation chambers (BDC), all with the same cylindrical shape and diameters, but with different lengths. Due to differences in length/volume, different BDC yield different magnitudes of blast intensity delivered to the cranium. The BDC terminates in a BDC-cranium interface (BDCCI), which directly contacts the scalp of the dorsal cranium of the rat. The BDCCI is fitted with an O-ring constructed of a soft gel-like material that forms a gentle seal with the scalp. The dorsum of the rat's head (shaved scalp) is lifted into place against the O-ring by use of an inflatable pillow positioned beneath the mandible that is inflated to 75 mm Hg to form the seal. The entire assembly of gun, BDC, BDCCI and the platform used to support the rat, is held vertically with the aid of two vertically adjustable stages, the upper one of which fixes the gun and neutralizes its safety, and the other of which allows accommodating different sizes (lengths) of BDCs.

Blast overpressure measurements. Measurements of blast pressure were made using a pressure transducer and charge amplifier (models 100P and 4601, respectively; Columbia Research Labs, Woodlyn, PA), with the output recorded using a D-A converter (Digidata 1200 data acquisition system, Axon Instruments). Typical

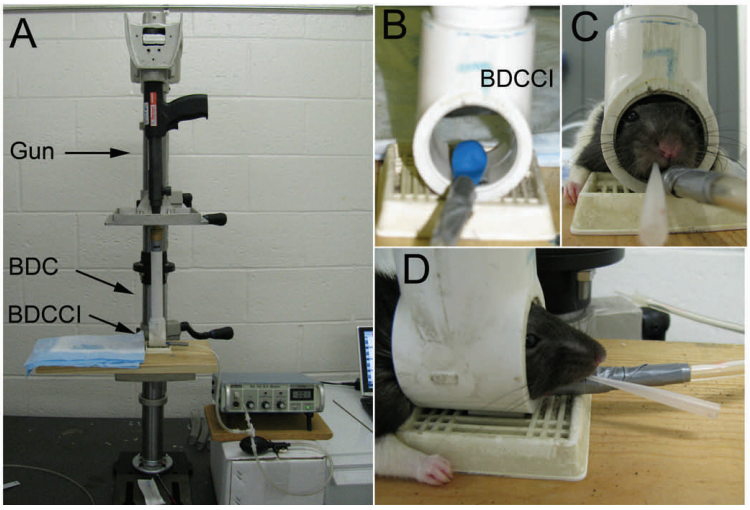


Fig 1. Cranium-only blast injury apparatus (COBIA). The entire apparatus (A) and 3 views of the BDCCI, with or without a rat in position (B–D), are shown. The view in B without a rat shows the inflatable pillow used to raise the rat's head to engage the O-ring of the BDCCI. The views in C and D show the anesthetized, intubated rat in position before blast. BDC, blast dissipation chamber; BDCCI, BDC-cranium interface.

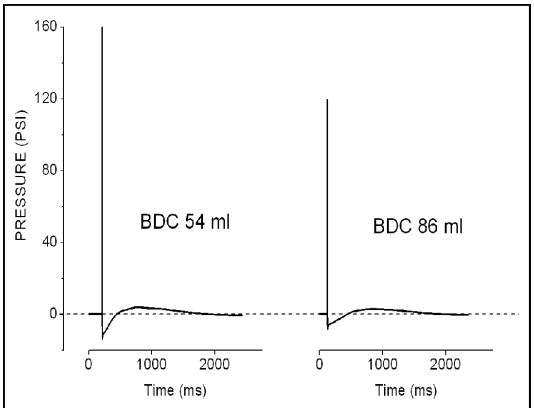


Fig 2. Blast pressure waveforms. Recordings shown at low temporal resolution of pressure waveforms obtained with 2 different BDCs, the 54 ml chamber and the 86 ml chamber (blast-to-scalp distance, 39.5 and 49.8 cm, respectively); power level #4; data acquisition sampling rate, 100 kHz.

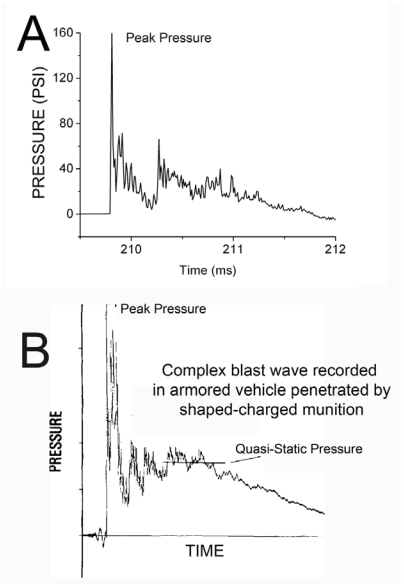


Fig 3. Blast pressure waveforms. A: Recording at high temporal resolution of early overpressure components, including initial peak and quasi-static components from COBIA. **B:** Published recording of blast wave recorded in armored vehicle penetrated by shaped charge munition (from citation #1).

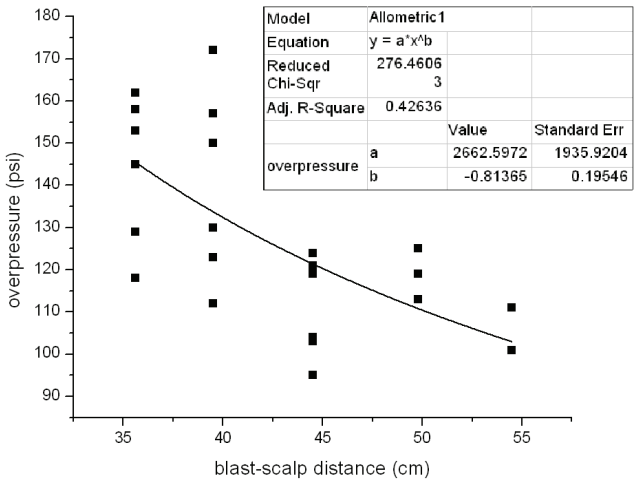


Fig. 4. Peak blast overpressures generated by COBIA with various sizes of BDC. Values shown are for power level 2 shots only, with values plotted against the blast-to-scalp distance. A least-squares fit of the data to a power function is also shown (solid line).

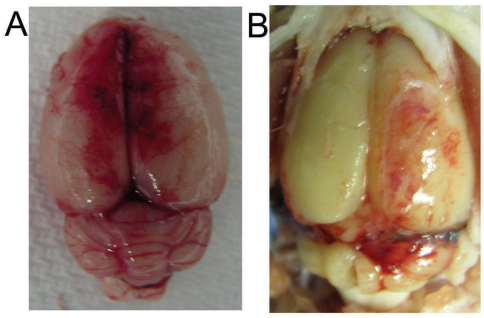


Fig 5. Gross pathology after blast-TBI. A,B: Surface views of brains following blast-TBI induced with BDC 54 ml (A) and 103 ml (B); blast-TBI was lethal in A but not in B; the rat in B was perfused after euthanasia 24 h post blast-TBI (rats 76 and 72).

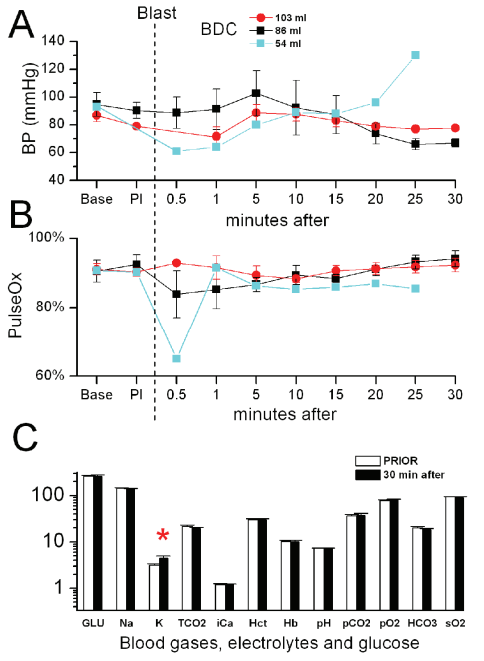


Fig 7. Physiological variables after blast-TBI. A,B: Systolic blood pressure (A) and pulse oxymetry (B) before and after blast-TBI induced with the BDCs indicated. **C:** Blood gases, electrolytes and glucose before and after blast-TBI; note logarithmic scale; the only significant difference was in serum potassium; mean values for 3 rats for 86 and 103 ml; 1 rat for 54 ml.

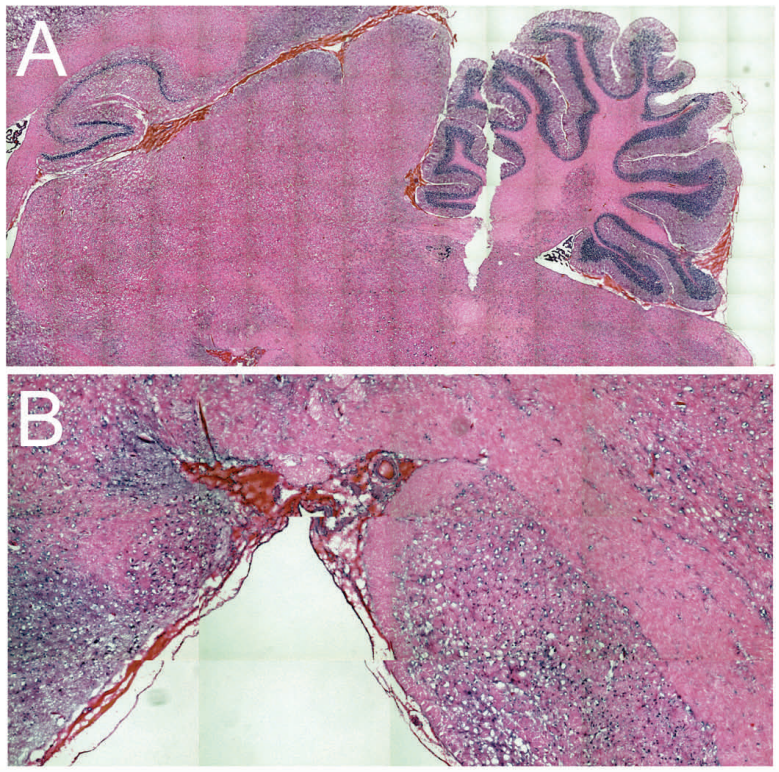


Fig 6. Histopathology after blast-TBI. A,B: Parasagittal sections of brain stained with hematoxilin and eosin, showing extensive subarachnoid hemorrhage; note eosinophilic neurons in brainstem; post-mortem fixation by immersion; rat 76, same as in Fig. 5A.

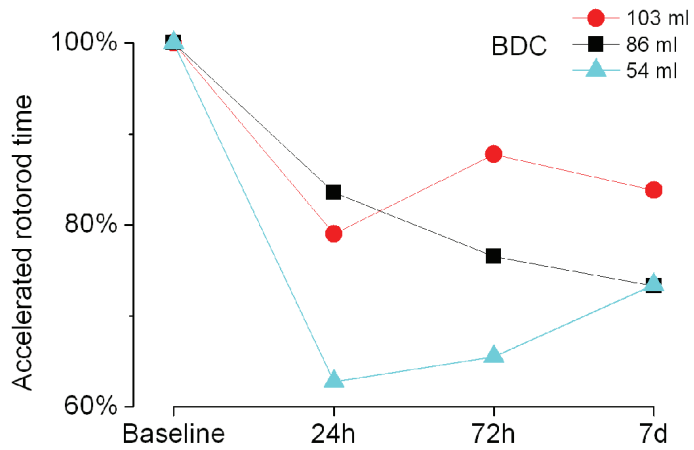
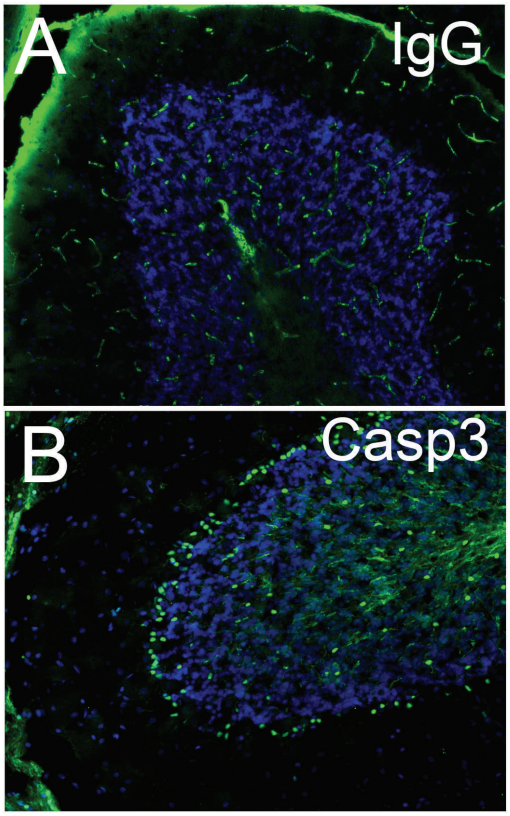


Fig 8. Accelerated Rotarod performance after blast-TBI. Performance on accelerated Rotarod before and after blast-TBI induced with the BDCs indicated, showing “dose-response” relationship for this outcome variable; the data shown are mean values for 3–7 rats per group.



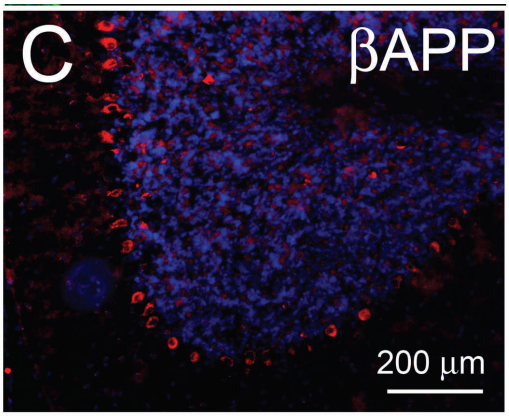


Fig 9. Immunohistochemistry after blast-TBI. A–C: Immunolabeling of cerebellum for IgG (A, green), activated caspase 3 (B, green) and β-amyloid precursor protein (β-APP) (C, red); in all cases, the nuclei were labeled with DAPI (blue); note the extensive capillary labeling with IgG in A, and the extensive Purkinje cell labeling for activated caspase 3 and β-APP in B and C; brains perfused to remove intravascular blood. Labeling of control uninjured brains showed no signal (data not shown).

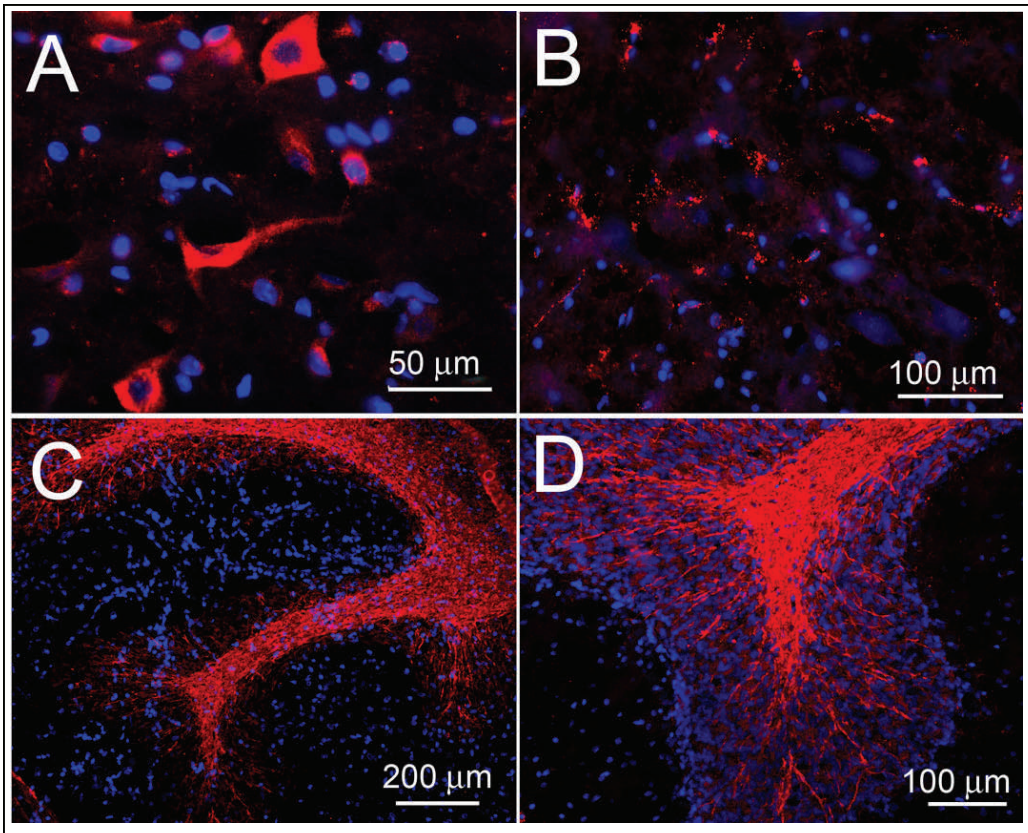


Fig 10. Immunohistochemistry after blast-TBI. A–D: Immunolabeling of brainstem (A,B) and cerebellum (C,D) for SUR1 (red); in all cases, the nuclei were labeled with DAPI (blue); note the extensive neuronal (A) and capillary (B) labeling in brainstem, and the extensive axonal labeling in the cerebellum (C,D). Labeling of control uninjured brains showed no signal (data not shown).

recordings of blast waveforms, with the transducer placed where the rat's head would be, show an initial large transient overpressure, followed by smaller transient under- and overpressures which are fully damped after ~2 sec (Fig. 2). At high temporal resolution, the initial overpressure is seen to be quite complex, including a " quasi-static" pressure component (Fig. 3A). Notably, the initial complex overpressure waveforms generated by the COBIA resemble closely the published recordings of blast overpressure waveforms recorded in an armored vehicle penetrated by shaped charge munitions (Fig. 3B). [1] Initial peak overpressure measurements recorded with different BDCs are given in Table 1 for power level 2 and level 4 shots. Individual measurements for level 2 shots are plotted in Fig. 4.

TABLE 1. Initial peak overpressures.

		PEAK BLAST OVERPRESSURE Mean values (n=3–5)	
volume of blast dissipation chamber (ml)	blast-to-scalp distance (cm)	Power Level 2 shot (PSI)	Power Level 4 shot (PSI)
39	35.6	144	–
54	39.5	140	195
69	44.5	111	–
86	49.8	119	155
103	54.5	104	127

Blast model. Adult male rats (250-300 gm) were studied. General anesthesia was induced (ketamine, 60 mg/kg and xylazine, 7.5 mg/kg, intraperitoneally). The tail artery was cannulated to obtain blood samples for analysis (i-STAT, Heska Corp, Fort Collins, CO) and to monitor blood pressure (Research grade invasive blood pressure monitor, BPM02, CyQ Scientific Instruments). The animals were intubated, but were allowed to ventilate air spontaneously. (The animals were intubated to prevent injury-induced airway collapse, and to facilitate rapid initiation of ventilatory support in the event of ventilatory arrest post-injury.) The rat was then positioned in the COBIA, and the inflatable pillow beneath the mandible was inflated to 75 mm Hg to elevate the head and to form a seal between the scalp and the o-ring of the BDCCI. A pulse oximeter (MouseOx, Starr Life Science Corp) was used to monitor heart rate and oxygen saturation. Blast injjry was induced, following which the pillow was deflated. Physiological variables were monitored for 30 min post-injury. In the event of a respiratory arrest, the endotrachial tube was connected to the ventilator and resuscitation was attempted for several minutes or until cardiac arrest.

To our knowledge, our current COBIA is the first of its kind, and as such is an important advance in the field of blast-TBI. Our approach to blast-TBI and our specific implementation of COBIA are deliberately conceptualized to direct blast forces selectively to the head, sparing lungs and other intrathoracic organs from direct blast

injury, and thereby minimiz ing transthoracic CNS effects and other injuries, as occur with area-blast chambers that act on the ent ire body. T his desi gn feature is particularly important for determining specific involvement of transcranial blast in blast-TBI, independent of transthoracic mechanisms.

Objectibe 1b – determining the threshold for lethality

Blast-TBI – mortality. Blast-T BI of high magnitude directed to the parieto-occipital region result ed in significant mortal ity (Table 2). Our data indicate that delivery of peak blast overpressures of 160 PSI to the scalp of the parieto-occipital region was associated with ~90% morta lity, whereas peak pressures <150 PSI were less likely to be fatal. By compari son, peak pressures of 30 PSI delivered to th e exposed dura after craniectomy (fluid percussion model of brain injury) are associated with very high mortality.[2,3] Together, these data suggest that the scalp plus sk ull introduc e a 5-fold attenuation of the blast overpressure.

When deat h occurred after blast injury, it occurred either immediately (<15 sec) or within 30 min. No delayed deaths beyon d these times were observed. With pow er level 4 shots, all sur vivors experienced prolonged apnea, whic h in s ome cases was accompanied by seizures (Table 2).

Subjects with immediate ear ly death (<15 sec) exhibi ted immediate respiratory arrest, followed by a few moments of seizur e-like activity involv ing hin dlimbs, follo wed by extension, especially of the tail, followed by cardiac arrest. V entilatory support was administered, resulting in several cases of successful resuscitation, but more often , resuscitation was uns uccessful. These rats were examined at necropsy (Fig. 5,6). Diffuse subarachnoid hemorrhages were evident over the dorsal and ventral surfaces of the brain, especially over t he cerebellum, and involving the brainstem, as reported in humans.[4] No gross untoward findings were observed involving the heart, lungs or thorax.

TABLE 2. Mortality, apnea and seizures

		MORTALITY		**APNEA / SEIZURE IN SURVIVORS**	
volume of blast dissipation chamber (ml)	blast-to-scalp distance (cm)	Level 2 shell (PSI)	Level 4 Shell (PSI)	Level 2 shell (PSI)	Level 4 Shell (PSI)
54	39.5	0%	75%	40%	100%
86	49.8	–	12%	–	100%
103	54.5	–	0%	–	75%

Subjects with delay ed early death (< 30 min) exhibited seemingly normal neurological and pulmonary function immediately after the injury, based on visual observation and physiological monitoring. However, 20-30 min post-injury, they exhibited frothy pulmonary exudate at the mouth that in at least one case was preceded

by systemic hypertension. Ventilatory support failed to resuscitate these rats. They were examined at necropsy and found to have spotty pulmonary hemorrhages, consistent with neurogenic pulmonary edema. They also had gross intracranials ubarachnoid hemorrhages similar to rats with immediate early deaths.

It is recognized that total body exposure to area-blast results in blast-TBI that is partly ameliorated by wearing chest armor, consistent with transt horacic delivery of a blast-induced force to the brain. However, the converse – blast-induced brain injury resulting in potentially fatal pulmonary dysfunction (neurogenic pulmonary edema) – is not widely appreciated. As the use of body armor becomes more prevalent, one can anticipate that blast-induced neurogenic pulmonary edema may become more prevalent.

Objectibe 1c – pathophysiological responses to sublethal blast

Blast-TBI – physiological variables. Blood pressure, pulse oxymetry (O_2 saturation), blood gases, electrolytes and glucose were measured for blast with BDC of 54, 86 and 103 ml, power level 4 shots (Fig. 7).

Only one rat survived the highest level blast. It showed showed the most severe changes in blood pressure and pulse oxymetry readings. Non-fatal levels of blast-TBI showed less severe alterations.

Blood gases, electrolytes and glucose measured 30 min after injury were largely unaffected, except for values of potassium, which we tentatively attribute to hemolysis of blood samples on drawing, rather than to injury.

Balst-TBI – neurobehavioral testing. Three neurobehavioral assessments were carried out, accelerated Rotarod, spontaneous rearing and beam walk. In general, the degree of impairment correlated with the blast severity (a "dose-response" effect). Data for accelerated Rotarod performance indicated sustained abnormal performance that lasted the entire week of testing (Fig. 8). Similar observations were made with the other two tests (not shown).

Blast-TBI – immunohistochemistry. In humans subjected to blast overpressure, a very important clinical manifestation of blast-TBI is edema due to capillary dysfunction, and neuronal cell death.

We used immunolabeling for IgG as an indicator of capillary dysfunction. Normal capillaries contain circulating IgG, but endothelial cells do not display intracellular uptake of IgG except under pathological conditions.[5] When examined 1 week after blast-TBI, following perfusion to remove intravascular contents, capillaries in the cerebellum showed widespread intracellular uptake of IgG in capillary endothelial cells, indicative of pathological involvement (Fig. 9A).

We used immunolabeling for activated caspase-3 as a marker of apoptotic death signaling, and β-amyloid precursor protein (β-APP), as a marker of neuronal injury. Both were markedly upregulated in Purkinje cells of the cerebellum 1 week after non-lethal blast TBI, indicative of severe pathological involvement (Fig. 9B,C).

OBJECTIVE 2: using this rat model, determine the specific role of the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel in blast-TBI, including testing whether block of SUR1 using glibenclamide would show a beneficial effect in blast-TBI.

Background. Several reports from this lab have established that the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel plays a critical role in various forms or CNS injury, including brain ischemia,[5,6] subarachnoid hemorrhage,[7] and brain[8] and spinal cord[9] contusive injury.[10] In these disease processes, the SUR1-regulated $NC_{a\text{-}ATP}$ channel plays a critical role in cellular (cytotoxic) edema, in formation of ionic and vasogenic edema, and in hemorrhagic transformation. Preliminary data presented with the original proposal demonstrated that the regulatory subunit of the channel was upregulated following blast-TBI produced by our original apparatus.

Introduction. Determining the specific role of the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel in direct blast-TBI entails several sub-objectives:

(**a**) assessing the time course and cellular localization for *de novo* upregulation of SUR1, the regulatory subunit of the channel;

(**b**) assessing the time course and cellular localization for *de novo* upregulation of TRPM4, the pore-forming subunit of the channel;

(**c**) assessing the effect of the SUR1 blocker, glyburide, on direct blast-TBI, by determining whether it shifts the apparent intensity-response relationship to the right

SUR1 in neurons, white matter and capillaries following blast-TBI. We have begun experiments to assess *de novo* upregulation of the regulatory subunit of the channel, SUR1, following direct blast-TBI induced by COBIA.

Non-lethal blast-TBI resulted in surprisingly widespread upregulation of SUR1 in neurons and capillaries of the brainstem (Fig. 10A,B). More surprising was the widespread upregulation in white matter bundles in cerebellum (Fig. 10C,D). The latter is a completely novel finding that signals severe pathological involvement of oligodendrocytes. It is likely that these severe changes in the cerebellum account for the vestibulomotor abnormalities recorded in these same rats.

TRPM4 in neurons, white matter and capillaries following blast-TBI. This activity has not yet been started.

Effect of the SUR1 blocker, glyburide, on outcome following blast-TBI. This activity has not yet been started.

OBJECTIVE 3: in normal human volunteers, determine the safety of oral glibenclamide as it might be used as prophylaxis against blast-TBI.

The SUR1 blocker, glyburide, in normal human volunteers. This activity has not yet been started.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

- Completion of development, construction and implementation of COBIA for production of reliable, repeatable, " dose-dependent" blast-TBI, independent of transthoracic mechanisms of blast injury to the brain.
- Using the latest versi on of COBIA, demons tration of a signific ant incidence of fatal neurogenic pulmonary edema, completely in dependent of transthoracic mechanisms of blast injury to the brain.
- Using the latest version of COBIA, demonstrati on of signific ant effects of blast-TBI on neurobehavioral function and on pathophysiological manifestations (IgG, caspase-3 and β-APP immunolabeling), independent of transthoracic mechanisms of blast injury to the brain.
- Using the latest version of COBIA, demonstrati on of signific ant effects of blast-TBI on upregulation of SUR1, the regulatory subunit of the SUR1-regulated $NC_{Ca\text{-}ATP}$ channel that has been widely im plicated in ischemic and traumatic injury to the CNS, further strengthening the rationale for prophylaxis and treatm ent using the SUR1 inhibitor, glyburide.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include: manuscripts, abstracts, presentations; patents and licenses applied for and/or issued; degrees obtained that are supported by this award; development of cell lines, tissue or serum repositories; infomatics such as databases and animal models, etc.; funding applied for based on work supported by this award; employment or research opportunities applied for and/or received based on experience/training supported by this award.

- Abstract submission to the upcoming Military Health Research Forum (MHRF) conference, to be held in Kansas City, Missouri August 31–September 3, 2009.

CONCLUSION: Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

Overall, the project is on track as planned in our original propos al. There are two ver y important results to date:

(i) completion of the development phase of COBIA, a nd beginning of experiments validating its utility for pr oduction of blast-TBI that reproduces numerous aspects of the pathophysiology of blast-TBI in humans

(ii) early demonstration of involvement of SUR1 in s econdary injury following blast-TBI, with the novel unprecedented fi nding of widespread upregulation of SUR1 in oligodendroc ytes of white matter, giving strong rationale to proceed with study of the SUR1 inhibitor, glyburide

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in *Science, Military Medicine*, etc.).

Reference List

(1) Stuhmiller J, Phillips Y, Richmond R. The physics and mechanisms of primary blast injury. In: Bellamy R, Zajtchuk R, eds. *Textbook of Military Medicine. Conventional Warfare: Ballistic, Blast, and Burn Injuries*.Washington, D.C.: Department of the Army, Office of the Surgeon General, Borden Institute; 1991. p. 241-70.

(2) Thompson HJ, Lifshitz J, Marklund N, Grady MS, Graham DI, Hovda DA, McIntosh TK. Lateral fluid percussion brain injury: a 15-year review and evaluation. *J Neurotrauma* 2005 January;22(1):42-75.

(3) Toth Z, Hollrigel GS, Gorcs T, Soltesz I. Instantaneous perturbation of dentate interneuronal networks by a pressure wave-transient delivered to the neocortex. *J Neurosci* 1997 November 1;17(21):8106-17.

(4) Bell RS, Vo AH, Neal CJ, Tigno J, Roberts R, Mossop C, Dunne JR, Armonda RA. Military traumatic brain and spinal column injury: a 5-year study of the impact blast and other military grade weaponry on the central nervous system. *J Trauma* 2009 April;66(4 Suppl):S104-S111.

(5) Simard JM, Yurovsky V, Tsymbalyuk N, Melnichenko L, Ivanova S, Gerzanich V. Protective effect of delayed treatment with low-dose glibenclamide in three models of ischemic stroke. *Stroke* 2009 February;40(2):604-9.

(6) Simard JM, Chen M, Tarasov KV, Bhatta S, Ivanova S, Melnitchenko L, Tsymbalyuk N, West GA, Gerzanich V. Newly expressed SUR1-regulated NC(Ca-ATP) channel mediates cerebral edema after ischemic stroke. *Nat Med* 2006 April;12(4):433-40.

(7) Simard JM, Geng Z, Woo SK, Ivanova S, Tosun C, Melnichenko L, Gerzanich V. Glibenclamide reduces inflammation, vasogenic edema, and caspase-3 activation after subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 2009 February;29(2):317-30.

(8) Simard JM, Kilbourne M, Tsymbalyuk O, Tosun C, Caridi J, Ivanova S, Keledjian K, Bochicchio G, Gerzanich V. Key role of sulfonylurea receptor 1 in progressive secondary hemorrhage following brain contusion. *J Neurotrauma* 2009 July 15.

(9) Simard JM, Tsymbalyuk O, Ivanov A, Ivanova S, Bhatta S, Geng Z, Woo SK, Gerzanich V. Endothelial sulfonylurea receptor 1-regulated NC Ca-ATP channels mediate progressive hemorrhagic necrosis following spinal cord injury. *J Clin Invest* 2007 August;117(8):2105-13.

(10) Simard JM, Woo SK, Bhatta S, Gerzanich V. Drugs acting on SUR1 to treat CNS ischemia and trauma. *Curr Opin Pharmacol* 2008 February;8(1):42-9.

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Abstract submission to the upcoming Military Health Research Forum (MHRF) conference, to be held in Kansas City, Missouri August 31–September 3, 2009.

SUPPORTING DATA: All figures and/or tables shall include legends and be clearly marked with figure/table numbers.

none

DIRECT CRANIAL BLAST, NOVEL RODENT MODEL OF BLAST TRAUMATIC BRAIN INJURY

J. Marc Simard,[1] Reed Kuehn,[2] Kaspar Keledjian,[1] Philippe F. Simard,[1] Svetlana Ivanova,[1] Grant Bocchicchio,[1] and Volodymyr Gerzanich[1]

[1]University of Maryland School of Medicine and [2]Walter Reed Army Medical Center

Background and Objectives: Blast-induced traumatic brain injury (blast-TBI) is commonly encountered on the modern battlefield and leads to severe morbidity and life\long adverse sequelae in warfighters. Unlike other causes of TBI, relatively little is known about the pathophysiology and secondary injury mechanisms involved in blast-TBI. Also, in blast-TBI, brain injury may be due to direct exposure of the cranium to the blast, or to indirect effects due to pulmonary injury, but the relative contribution of each is unclear. These and other deficiencies in understanding are due largely to the absence of satisfactory animal models of direct cranial blast. Here, we describe a novel model of blast-TBI in which the cranium of anesthetized rats is directly exposed to a blast with minimal exposure of the rest of the body.

Brief Description of Methodologies: Blasts were created by firing .22 caliber blanks (130 mg gunpowder) to accelerate a piston inside the barrel of the gun. The gun barrel was connected to one of a variety of cylindrical blast dissipation chambers, the other end of which had a 25-mm aperture that was positioned directly over the dorsal cranium of the rat. The system produced peak blast pressures of 120–180 psi, with negative after pressures ~10% of peak pressures. Rats were anesthetized, intubated and instrumented for blood pressure, blood gas sampling, and pulse oximetry. Immunolabeling for IgG following perfusion to remove intravascular contents was used to assess capillary endothelial injury and vasogenic edema. Immunolabeling for beta amyloid precursor protein was used to confirm neuronal injury.

Results to Date: Physiological monitoring during blasts showed modest decreases in O2 saturation (~5% decrease) that lasted 15–20 minutes before normalizing, consistent with minimal pulmonary injury. A "dose-response" relating blast intensity and severity of injury was noted. At 24–48-hour post-blast-TBI, rats exhibited reduced spontaneous activity, abnormal beam walk, and inability to maintain performance on RotaRod. Gross examination at necropsy of brains with stronger blasts revealed subarachnoid hemorrhages in the interhemispheric fissure, over the parietal cortex and over the cerebellum, as well as intraventricular hemorrhages and petechial hemorrhages in deep brain regions. With stronger blasts, widespread immunolabeling for IgG was apparent in numerous brain regions. IgG endocytosis indicative of capillary dysfunction was found in capillary endothelial cells, and foci of IgG extravasation indicative of vasogenic edema were found in many areas. In addition, beta amyloid precursor protein was diffusely upregulated in neurons and axons in hippocampus, cerebellum, and other brain regions.

Conclusions: The novel blast-TBI model described here, with blast energy directed to the cranium, reproduced many of the features encountered in human blast-TBI, including widespread capillary dysfunction, vasogenic edema, petechial hemorrhages, and diffuse upregulation of beta amyloid precursor protein.

Impact Statement: The novel blast-TBI model described here will advance molecular and cellular understanding of secondary injury mechanisms induced by blast injury to the brain.

This work was supported by the U.S. Army Medical Research and Materiel Command under W81XWH-08-2-0157.